

### NEB-232.ST25.txt SEQUENCE LISTING

<110> Lunnen, Keith Davis, Theodore Wilson, Geoffrey

<120> Method for Cloning and Expression of SbfI Restriction Endonnuclease and SbfI Methylase in E. coli

<130> NEB-232

<140> 10/668,047 <141> 2003-09-22

<141> 2003-09-22

<150> 10/668,047 <151> 2003-09-22

<160> 57

<170> PatentIn version 3.2

<210> 1

<211> 1461

<212> DNA

<213> streptomyces species Bf-61

<220>

<221> CDS <222> (1)..(1461)

<400> 1

aaa ctg gac ccc act act caa gcg gtg cta ggg cag ttc ttc act ccc 96
Lys Leu Asp Pro Thr Thr Gln Ala Val Leu Gly Gln Phe Phe Thr Pro

atg aag gcc gcc acg ctg atg gct tca atg ctt cgg gtc gat gat ctc

Met Lys Ala Ala Thr Leu Met Ala Ser Met Leu Arg Val Asp Asp Leu

35 40 45

cgc gga acg gtg cgg gtg ctc gac cca gga gct ggt gtc ggg tct ctg
Arg Gly Thr Val Arg Val Leu Asp Pro Gly Ala Gly Val Gly Ser Leu
50 60

acc gct gcc ctc gtc gat cgg ctg cat act gaa cgc ccc gac gtt gcg
Thr Ala Ala Leu Val Asp Arg Leu His Thr Glu Arg Pro Asp Val Ala
65 70 75 80

gtc cac gta gtt gcc gtg gaa acc gac ccc ttt gtc gtg cct tac ctg
Val His Val Val Ala Val Glu Thr Asp Pro Phe Val Val Pro Tyr Leu

85 90 95

cgc gcc acc ctg gag gaa tgt cgg aac gct tac ggc atc tcc tac gac 336
Arg Ala Thr Leu Glu Glu Cys Arg Asn Ala Tyr Gly Ile Ser Tyr Asp
100 105 110

cta gtc gag ggc gac tat ttg ctt aac caa ggg gcc aag ctg gat ggc 384
Leu Val Glu Gly Asp Tyr Leu Leu Asn Gln Gly Ala Lys Leu Asp Gly
115 120 125

Page 1

ccg Pro	ttc Phe 130	gat Asp	ctt Leu	gta Val	att Ile	gct Ala 135	aat Asn	cct Pro	ccc Pro	tac Tyr	gga Gly 140	aag Lys	ctt Leu	gct Ala	tca Ser	432
gat Asp 145	tcg Ser	ctg Leu	gcg Ala	cgg Arg	ctt Leu 150	gca Ala	acg Thr	aca Thr	gcg Ala	cgt Arg 155	gcc Ala	gtc val	gat Asp	gta val	ccg Pro 160	480
aac Asn	gtt .val	tac Tyr	gtg Val	gcc Ala 165	ttc Phe	tgg Trp	gtg Val	cga Arg	gca Ala 170	gtc Val	att Ile	tcg Ser	ctc Leu	aaa Lys 175	gag Glu	528
cag Gln	ggg Gly	cgg Arg	ggg Gly 180	gtt Val	ttc Phe	att Ile	gtt val	cct Pro 185	cga Arg	tct Ser	tgg Trp	gcg Ala	aac Asn 190	ggg Gly	cct Pro	576
tac Tyr	tat Tyr	cgt Arg 195	caa Gln	ttt Phe	cgc Arg	cat His	tgg Trp 200	ctg Leu	atg Met	acc Thr	gcg Ala	gta Val 205	agt Ser	ctc Leu	gat Asp	624
ata Ile	ctt Leu 210	cat His	gtg val	ttc Phe	gaa Glu	agt Ser 215	aga Arg	acc Thr	aaa Lys	gta Val	ttt Phe 220	gcg Ala	gac Asp	acg Thr	aag Lys	672
gta Val 225	aag Lys	caa Gln	gag Glu	aat Asn	gtc Val 230	atc Ile	gtt Val	gct Ala	ttc Phe	agt Ser 235	gtg val	agg Arg	ccg Pro	caa Gln	agc Ser 240	720
tct Ser	agt Ser	gtg Val	gtc val	ctt Leu 245	tct Ser	agg Arg	tcg Ser	gtc Val	gca Ala 250	cat His	gga Gly	gaa Glu	gag Glu	tcg Ser 255	atc Ile	768
gca Ala	agt Ser	tct Ser	gtg Val 260	ccg Pro	ttt Phe	tct Ser	gcg Ala	ctt Leu 265	gtt Val	cat His	gat Asp	gaa Glu	gac Asp 270	gat Asp	gac Asp	816
aaa Lys	atc Ile	gtg Val 275	cac His	ttc Phe	gcg Ala	gaa Glu	agc Ser 280	gca Ala	tcg Ser	gtg Val	ccc Pro	tcg ser 285	gcg Ala	gcg Ala	agg Arg	864
ttt Phe	act Thr 290	ctc Leu	gct Ala	gat Asp	ctc Leu	ggc Gly 295	atc Ile	ggt Gly	gta Val	agt Ser	acg Thr 300	gga Gly	aag Lys	gtt val	gtt Val	912
gat Asp 305	ttt Phe	cgc Arg	aat Asn	cgt Arg	cag Gln 310	tat Tyr	ttg Leu	acc Thr	gat Asp	aac Asn 315	ctg Leu	gat Asp	gct Ala	tca Ser	ggc Gly 320	960
gtt Val	gtg Val	ccc Pro	atg Met	gtt Val 325	tat Tyr	cag Gln	tca Ser	aac Asn	att Ile 330	cga Arg	tct Ser	ggt Gly	aaa Lys	att Ile 335	gat Asp	1008
tgg Trp	cct Pro	cag Gln	gtg Val 340	ggt Gly	gcg Ala	agg Arg	aag Lys	cct Pro 345	caa Gln	gga Gly	ttt Phe	gtt Val	gcg Ala 350	gtc Val	gaa Glu	1056
gat Asp	gta Val	gca Ala 355	cta Leu	cgt Arg	caa Gln	ctt Leu	ctc Leu 360	ccg Pro	caa Gln	ggg Gly	tcg Ser	tat Tyr 365	gtt Val	gtt Val	gtg Val	1104
aaa Lys	cgg Arg	caa Gln	acg Thr	gcg Ala	aaa Lys	gag Glu	gac Asp	cgt Arg	Arg	cgt Arg Page	٧a١	atc Ile	gct Ala	gcg Ala	gtc Val	1152

370				3/5					380					
tgg gac Trp Asp 385	ggg g Gly A	cc agc la Ser	agg Arg 390	gtt Val	gcg Ala	ctc Leu	gac Asp	aat Asn 395	aaa Lys	acg Thr	aac Asn	tat Tyr	ttg Leu 400	1200
cat gaa His Glu	tct c	aa cga In Arg 405	ccg Pro	ctt Leu	gag Glu	aaa Lys	aat Asn 410	gtg Val	gcc Ala	cgc Arg	ggc Gly	ctc Leu 415	мет	1248
ctt tgg Leu Trp	Leu A	ac tcg sn Ser 20	act Thr	gtg Val	ttg Leu	gat Asp 425	cag Gln	tat Tyr	ttc Phe	cga Arg	gcc Ala 430	ttt Phe	tcc Ser	1296
ggg cat Gly His	acc ca Thr G 435	ag gtg In Val	aac Asn	gct Ala	ggc Gly 440	gat Asp	cta Leu	cgc Arg	cgg Arg	ctt Leu 445	ccg Pro	ttc Phe	ctc Leu	1344
tgt cgc Cys Arg 450	gag ga Glu As	ac cta sp Leu	att Ile	ctt Leu 455	ctc Leu	gct Ala	aag Lys	gtc Val	gtt Val 460	ccc Pro	gat Asp	ggc Gly	ctg Leu	1392
cct gat Pro Asp 465	cag ga Gln G	ag acg lu Thr	ttg Leu 470	gat Asp	gcc Ala	gtg Val	gtg Val	gcc Ala 475	aga Arg	ctc Leu	ttc Phe	tgt Cys	gag Glu 480	1440
att ccg	gaa to Glu So	ct gcc er Ala 485	tcg Ser	tga										1461
<212> PI	86 RT	omyces	spec	cies	Bf-6	51							,	
<400> 2														
Met His   1	Pro I	le Ala 5	Ser	Thr	Glu	Thr	Arg 10	Arg	Gln	Ala	Ala	Leu 15	Gly	
Lys Leu /	Asp Pi 20		Thr	Gln	Ala	Va1 25	Leu	Gly	Gln	Phe	Phe 30	Thr	Pro	
Met Lys /	41a A <sup>*</sup> 35	la Thr	Leu	Met	Ala 40	Ser	Met	Leu	Arg	∨a1 45	Asp	Asp	Leu	
Arg Gly 50	Thr Va	al Arg	val	Leu 55	Asp	Pro	Gly	Αla	G]y 60	val	Gly	Ser	Leu	
								<b>63</b>	٨٣٥	D.::-	4	Va.1	475	
Thr Ala / 65	Ala Le	eu Val	Asp 70	Arg	Leu	HIS	Thr	75	Arg	Pro	ASP	vai	80	
~=			70					75					80	

Arg Ala Thr Leu Glu Glu Cys Arg Asn Ala Tyr Gly Ile Ser Tyr Asp Page 3

110

Leu Val Glu Gly Asp Tyr Leu Leu Asn Gln Gly Ala Lys Leu Asp Gly
115 120 125 Pro Phe Asp Leu Val Ile Ala Asn Pro Pro Tyr Gly Lys Leu Ala Ser 130 135 140 Asp Ser Leu Ala Arg Leu Ala Thr Thr Ala Arg Ala Val Asp Val Pro 145 150 155 160 Asn Val Tyr Val Ala Phe Trp Val Arg Ala Val Ile Ser Leu Lys Glu 165 170 175 Gln Gly Arg Gly Val Phe Ile Val Pro Arg Ser Trp Ala Asn Gly Pro 180 185 190 Tyr Tyr Arg Gln Phe Arg His Trp Leu Met Thr Ala Val Ser Leu Asp Ile Leu His Val Phe Glu Ser Arg Thr Lys Val Phe Ala Asp Thr Lys 210 220 Val Lys Gln Glu Asn Val Ile Val Ala Phe Ser Val Arg Pro Gln Ser Ser Ser Val Val Leu Ser Arg Ser Val Ala His Gly Glu Glu Ser Ile 245 250 255 Ala Ser Ser Val Pro Phe Ser Ala Leu Val His Asp Glu Asp Asp Asp 260 265 270 260 Lys Ile Val His Phe Ala Glu Ser Ala Ser Val Pro Ser Ala Ala Arg 275 280 285 Phe Thr Leu Ala Asp Leu Gly Ile Gly Val Ser Thr Gly Lys Val Val 290 295 300 Asp Phe Arg Asn Arg Gln Tyr Leu Thr Asp Asn Leu Asp Ala Ser Gly Val Val Pro Met Val Tyr Gln Ser Asn Ile Arg Ser Gly Lys Ile Asp 325 330 335 Trp Pro Gln Val Gly Ala Arg Lys Pro Gln Gly Phe Val Ala Val Glu 345

								NI	EB-2	32.s <sup>-</sup>	Γ25.1	txt					
Asp	val	Ala 355	Leu	Arg	Gln	Leu	Leu 360	Pro	Gln	Gly	Ser	Tyr 365	Val	val	Val		
Lys	Arg 370	Gln	Thr	Ala	Lys	Glu 375	Asp	Arg	Ąrg	Arg	val 380	Ile	Ala	Ala	Val		
Trp 385	Asp	Gly	Ala	Ser	Arg 390	٧al	Ala	Leu	Asp	Asn 395	Lys	Thr	Asn	Tyr	Leu 400		
His	Glu	Ser	Gln	Arg 405	Pro	Leu	Glu	Lys	Asn 410	٧a٦	Ala	Arg	Gly	Leu 415	Met		
Leu	Trp	Leu	Asn 420	Ser	Thr	val	Leu	Asp 425	Gln	Tyr	Phe	Arg	Ala 430	Phe	Ser		
Gly	His	Thr 435	Gln	val	Asn	Ala	Gly 440	Asp	Leu	Arg	Arg	Leu 445	Pro	Phe	Leu		
Cys	Arg 450	Glu	Asp	Leu	Ile	Leu 455	Leu	Ala	Lys	val	val 460	Pro	Asp	Gly	Leu		-
Pro 465	Asp	Gln	Glu	Thr	Leu 470	Asp	Ala	val	val	Ala 475	Arg	Leu	Phe	Cys	Glu 480		
Ile	Pro	Glu∕	Ser	Ala 485	Ser												
<210 <211 <212 <213	L>	3 972 ONA strep	otomy	/ces	spec	ies	Bf-6	51									
<220 <222 <222	l> (	DS (1)	. (972	?)													
<400 gtg Met 1	aac	agc	agt Ser	gac Asp 5	ggc Gly	atc Ile	gac Asp	gga Gly	acg Thr 10	gta Val	gcg Ala	agc Ser	atc Ile	gat Asp 15	act Thr	•	48
gcg Ala	cgg Arg	gcg Ala	ctg Leu 20	cta Leu	aag Lys	cgt Arg	ttt Phe	ggg Gly 25	ttt Phe	gac Asp	gcg Ala	caa Gln	cga Arg 30	tat Tyr	aac Asn		96
gtc Val	cgt Arg	agc Ser 35	gct Ala	gtg Val	aca Thr	ttg Leu	ctc Leu 40	gcg Ala	ctt Leu	gcc Ala	ggt Gly	ttg Leu 45	aag Lys	ccg Pro	gga Gly	1	L44
gat Asp	cgc Arg 50	tgg Trp	gtt Val	gac Asp	tcg Ser	acc Thr 55	act Thr	cca Pro	cgc Arg	ctt Leu	ggc Gly 60	gtt Val	cag Gln	aag Lys	atc Ile	1	L92

	•		•															
	•					•												
at Me 65	g ga t As	c p	tgg Trp	tcc Ser	aac	gag Glu 70	cat His	tgg Trp	qcc	aag	32.ST ccg Pro 75	tac	gcc	acc Thr	gga Gly	agt Ser 80	;	240
cg Ar	a ga g Gl	a u .	gat Asp	ttc Phe	cgt Arg 85	aag Lys	aag Lys	acg Thr	ctt Leu	cgg Arg 90	cag Gln	tgg Trp	gtt Val	gat Asp	aat Asn 95	ggc Gly	ì	288
tt Ph	c gc e Al	c a	gta Val	ctt Leu 100	aat Asn	gcg Ala	gac Asp	aat Asn	tta Leu 105	aac Asn	atc Ile	gcc Ala	acg Thr	aac Asn 110	tcg Ser	cag Gln		336
ct Le	c aa u As	n	gag Glu 115	tac Tyr	tgc Cys	ttg Leu	tct Ser	gac Asp 120	gaa Glu	gca Ala	tta Leu	cag Gln	gcg Ala 125	cta Leu	agg Arg	gca Ala		384
ta Ty	t gg r Gl 13	y '	acg Thr	gaa Glu	ggc Gly	ttc Phe	gag Glu 135	gaa Glu	tct Ser	ctt Leu	gta Val	gtc Val 140	ttt Phe	ctt Leu	gat Asp	gaa Glu	•	432
gc Al 14	a Se	g r	aag Lys	gcg Ala	gtt Val	aaa Lys 150	gcg Ala	cga Arg	gcg Ala	gaa Glu	gct Ala 155	ctc Leu	cag Gln	gct Ala	gcg Ala	atg Met 160	•	480 .
at Il	c tc e Se	t r	gtc Val	gat Asp	ctc Leu 165	cct Pro	ggt Gly	ggc Gly	gag Glu	gaa Glu 170	ttt Phe	ctg Leu	ctc Leu	tcg Ser	cct Pro 175	gcc Ala		528
gg G1	g ca y Gl	g n	aat Asn	cca Pro 180	ttg Leu	ctg Leu	aag Lys	aag Lys	atg Met 185	gtc Val	gaa Glu	gag Glu	ttt Phe	gtg Val 190	ccg Pro	cga Arg		576
tt Ph	t gc e Al	a	cct Pro 195	cgc Arg	tcg Ser	acg Thr	gtg Val	ctc Leu 200	tac Tyr	ctc Leu	ggg Gly	gat Asp	act Thr 205	cgt Arg	gga Gly	aag Lys	(	624
ca Hi	t tc s Se 21	r	cta Leu	ttc Phe	gaa Glu	cga Arg	gag Glu 215	atc Ile	ttt Phe	gaa Glu	gag Glu	gtg Val 220	ctc Leu	ggc Gly	ctg Leu	act Thr	(	672
tt Ph	e Ās	c p	ccc Pro	cat His	ggt Gly	cga Arg 230	atg Met	ccg Pro	gac Asp	ctt Leu	att Ile 235	ctc Leu	cat His	gac Asp	gaa Glu	gtt Val 240	•	720
cg Ar	t gg g G1	g j	tgg Trp	ctt Leu	ttc Phe 245	ctt Leu	atg Met	gag Glu	gcc Ala	gtg Val 250	aaa Lys	agt Ser	aaa Lys	ggt Gly	ccg Pro 255	ttt Phe	•	768
ga As	t ga p Gl	g (	gag Glu	cgg Arg 260	cat His	cgc Arg	agc Ser	ctg Leu	caa Gln 265	gag Glu	cta Leu	ttc Phe	gtt Val	aca Thr 270	cct Pro	tca Ser	;	816
gc Al	g gg a Gl	y I	cta Leu 275	att Ile	ttt Phe	gta Val	aac Asn	tgc Cys 280	ttt Phe	gaa Glu	aat Asn	cgt Arg	gag Glu 285	tcg Ser	atg Met	cgt Arg	;	864
ca G1	g tg n Tr 29	p⊢	ctc Leu	cct Pro	gag Glu	ctg Leu	gct Ala 295	tgg Trp	gaa Glu	act Thr	gag Glu	gcg Ala 300	tgg Trp	gta Val	gcg Ala	gaa Glu	!	912
ga As 30	p Pr	a ,	gac Asp	cat His	ctg Leu	att Ile 310	cac His	ctt Leu	aac Asn	Gly	tct Ser 315 Page	Arg	ttt Phe	ctt Leu	ggg Gly	ccg Pro 320	!	960

tac gaa cgt tag Tyr Glu Arg

<210> 323 <211> <212> PRT

<400>

streptomyces species Bf-61

Met Asn Ser Ser Asp Gly Ile Asp Gly Thr Val Ala Ser Ile Asp Thr 1 10 15

Ala Arg Ala Leu Leu Lys Arg Phe Gly Phe Asp Ala Gln Arg Tyr. Asn 20 25 30

val Arg Ser Ala Val Thr Leu Leu Ala Leu Ala Gly Leu Lys Pro Gly

Asp Arg Trp Val Asp Ser Thr Thr Pro Arg Leu Gly Val Gln Lys Ile 50 60

Met Asp Trp Ser Gly Glu His Trp Ala Lys Pro Tyr Ala Thr Gly Ser 65 70 75 80

Arg Glu Asp Phe Arg Lys Lys Thr Leu Arg Gln Trp Val Asp Asn Gly
85 90 95

Phe Ala Val Leu Asn Ala Asp Asn Leu Asn Ile Ala Thr Asn Ser Gln

Leu Asn Glu Tyr Cys Leu Ser Asp Glu Ala Leu Gln Ala Leu Arg Ala 115 120 125

Tyr Gly Thr Glu Gly Phe Glu Glu Ser Leu Val Val Phe Leu Asp Glu 130 135 140

Ala Ser Lys Ala Val Lys Ala Arg Ala Glu Ala Leu Gln Ala Ala Met 145 150 155 160

Ile Ser Val Asp Leu Pro Gly Gly Glu Glu Phe Leu Leu Ser Pro Ala 165 170 175

Gly Gln Asn Pro Leu Leu Lys Lys Met Val Glu Glu Phe Val Pro Arg

Phe Ala Pro Arg Ser Thr Val Leu Tyr Leu Gly Asp Thr Arg Gly Lys 195 200 205 Page 7

His Ser Leu Phe Glu Arg Glu Ile Phe Glu Glu Val Leu Gly Leu Thr 210 215 220

Phe Asp Pro His Gly Arg Met Pro Asp Leu Ile Leu His Asp Glu Val 225 230 235 240

Arg Gly Trp Leu Phe Leu Met Glu Ala Val Lys Ser Lys Gly Pro Phe 245 250 255

Asp Glu Glu Arg His Arg Ser Leu Gln Glu Leu Phe Val Thr Pro Ser 260 265 270

Ala Gly Leu Ile Phe Val Asn Cys Phe Glu Asn Arg Glu Ser Met Arg 275 280 285

Gln Trp Leu Pro Glu Leu Ala Trp Glu Thr Glu Ala Trp Val Ala Glu 290 295 300

Asp Pro Asp His Leu Ile His Leu Asn Gly Ser Arg Phe Leu Gly Pro 305 310 315 320

Tyr Glu Arg

<210> 5 <211> 30

<212> PRT

<213> unknown

<220>

<223> SbfI Endonuclease

<400> 5

Ser Asp Gly Ile Asp Gly Thr Val Ala Ser Ile Asp Thr Ala Arg Ala 1 5 10 15

Leu Lys Arg Phe Gly Phe Asp Ala Gln Arg Tyr Asn Val 20 25 30

<210> 6

<211> 31

<212> PRT

<213> Unknown

<220>

<223> SbfI Endonuclease

<400> 6

Val Glu Glu Phe Val Pro Arg Phe Ala Pro Arg Ser Thr Val Leu Tyr Page 8

7 21 <210> <211> <212> DNA <213> unknown

<220> <223> primer

<400> 7 atgaactccg acggcatcga c

5

<210> 8

17 <212> DNA <213> unknown

<211>

<220> <223> primer

<220> <221> <222> misc\_feature (3)..(3)

<223> n = G,A,C or T

<220> misc\_feature

<221> <222> (6)..(6)<223> y = c or t

<220>

<221> <222> misc\_feature

(9)..(9)<223> r = a or g

<220>

misc\_feature (12)..(12) n = g,a,c or t <221>

<222> <223>

<400> 8

aanacyaart cnaccat

<210> 9

21 <211> <212> DNA

<213> unknown

<220>

<223> primer

<400> 9

atgaacagcg acggcatcga c

21

17

21

```
<210>
       10
<211>
       20
<212>
       DNA
<213> unknown
<220>
<223> primer
<220>
<221>
      misc_feature
<222> (3)..(3)
<223> s = g or c
<220>
<221> misc_feature
<222> (15)..(15)
<223> s = g or c
<400> 10
                                                                               20
aasaccaact cctcsaccat
       11
27
<210>
<211>
<212>
       DNA
<213>
      unknown
<220>
<223> primer
<400> 11
                                                                               27
ggagccatac agagagcgat ttattcg
<210> 12
<211> 27
<212> DNA
<213> unknown
<220>
<223> primer
<400> 12
                                                                               27
ttgaaatcga attaataagt ctggatg
<210>
       13
<211>
       24
<212>
      DNA
<213> unknown
<220>
<223>
       primer
<400> 13
                                                                               24
ccagtccatg atcttctgaa cgcc
<210>
       14
<211>
       24
<212> DNA
```

<213>	unknown	Neb Estistes exc	
<220> <223>	primer		
<400> cttcgg	14 cagt gggttgataa	tggc	24
<210> <211> <212> <213>	15 24 DNA unknown		
<220> <223>	primer		
<400> agggag	15 atcg acagagatca	tcgc	24
<210> <211> <212> <213>	16 24 DNA unknown		
<220> <223>	primer		
<400> tactgc	16 gcgg gcgctgctaa	agcg	24
<210> <211> <212> <213>	17 18 DNA unknown		
<220> <223>	primer		
	17 acct tcatccgg		18
<210> <211> <212> <213>	18 24 DNA unknown		
<220> <223>	primer		
<400> agggag	18 atcg acagagatca	tcgc	24
<210> <211> <212> <213>	19 24 DNA unknown		
<220>			

#### NFR-232.ST25.txt

<223>	primer		
<400>	19		
	gcgg gcgctgctaa	agcg	24
<210> <211>	20 24		
<212> <213>	DNA unknown		
<220> <223>	primer		
<400> agcgga	20 taac aatttcacac	agga	24
<210> <211>	21	·	
<211> <212> <213>	24 DNA unknown		
<220> <223>	primer		
<400> cgccag	21 ggtt ttcccagtca	cgac	24
<210>	22		
<211> <212> <213>	24 DNA unknown		
<220> <223>	primer		
<400> ccagtc	22 catg atcttctgaa	cgcc	24
<210>	23		
<211> <212>	36 DNA		
<213>	unknown	•	
<220> <223>	primer		
<400> tggggc	23 gaat tcccagtcca	tgatcttctg aacgcc	36
<210> <211>	24 25	,	
<212> <213>	DNA unknown		
<220> <223>	primer		
<100>			

tggggc	aagc ttgatcaggt	ccgtg	NEB-232.ST25.txt	25
<210> <211> <212> <213>	25 28 DNA unknown			
<220> <223>	primer			
<400> tggggca	25 aagc ttcgcctgct	ggttgacc		28
<210> <211> <212> <213>	26 28 DNA unknown			
<220> <223>	primer			
<400> tgtgggg	26 gcaa gcttcgcccc	ggtcgtcc		28
<210> <211> <212> <213>	27 27 DNA unknown			
<220> <223>	primer			
<400> tggggca	27 aagc ttctgcgatc	cgctgcc		27
<210> <211> <212> <213>	28 30 DNA unknown			
<220> <223>	primer			
<400> tggggca	28 aagc ttcgttggcg	gtgctcccgc		30
<210> <211> <212> <213>	29 24 DNA unknown			
<220> <223>	primer			
<400>	29 tgtc acagcgctac	ggac		24

210	7.0	NED ESEISTESTENC	
<210><211><212><213>	30 24 DNA unknown	•	
<220> <223>	primer		
<400> gatcca	30 acac agtcgagttc	aacc	24
<210> <211> <212> <213>	31 24 DNA unknown		
<220> <223>	primer		
<400> aacggc	31 aaac ggcgaaagag	gacc	24
<210> <211> <212> <213>	32 24 DNA unknown		
<220> <223>	primer		
<400> cctcate	32 gctt tggttgaact	cgac	24
<210> <211> <212> <213>	33 24 DNA unknown		
<220> <223>	primer		
<400> tcgttg	33 cttt cagtgtgagg	ccgc	24
	34 24 DNA unknown		•
<220> <223>	primer		
<400> cgttgtg	34 gccc atggtttatc	agtc	24
<210> <211>	35 24		

Page 14

```
NEB-232.ST25.txt
```

<213> unknown <220> <223> primer <400> 35 24 ctcttgcttt accttcgtgt ccgc <210> 36 <211> <212> 30 PRT <213> unknown <220> R.SbfI <223> <400> 36 Ser Asp Gly Ile Asp Gly Thr Val Ala Ser Ile Asp Thr Ala Arg Ala 1 5 10 15 Leu Leu Lys Arg Phe Gly Phe Asp Ala Gln Arg Tyr Asn Val 20 25 30 37 33 <210> <211> <212> PRT <213> unknown <220> <223> R.SbfI <400> Met Asn Ser Ser Asp Gly Ile Asp Gly Thr Val Ala Ser Ile Asp Thr  $1 \hspace{1cm} 10 \hspace{1cm} 15$ Ala Arg Ala Leu Leu Lys Arg Phe Gly Phe Asp Ala Gln Arg Tyr Asn 20 25 30 ٧a٦ <210> 38 <211> 24 <212> DNA <213> unknown <220> <223> primer <400> 38 24 ggccacgtaa acgttcggta catc

Page 15

39 24

<210> <211>

```
<212> DNA
<213>
       unknown
<220>
<223>
      primer
<400> 39
                                                                          24
tcatttcgct caaagagcag gggc
<210>
       40
       27
<211>
<212>
       DNA
<213>
      unknown
<220>
<223>
       primer
<400> 40
                                                                          27
ggagccatac agagagcgat ttattcg
<210>
       41
       27
<211>
<212>
      DNA
<213>
      unknown
<220>
<223>
       primer
<400> 41
                                                                          27
ttgaaatcga attaataagt ctggatg
<210>
       42
57
<211>
<212>
       DNA
<213>
       unknown
<220>
<223>
       primer
<400> 42
                                                                          57
ttccgggatc cggaggttta aaatatgact aagcggcaac acaattacct atatctc
       43
<210>
<211>
       48
<212>
      DNA
<213>
      unknown
<220>
<223>
       primer
ttgccgcatg cgtcgttaca ttagctgcaa actctgattg attatttc
                                                                          48
<210>
       44
<211>
       63
<212>
       DNA
<213>
      unknown
```

<220> <223>	primer					
<400> tggctg	44 cagg gatccggagg	tttaaaatat	gaacagcagt	gacggcatcg	acggaacggt	60
agc						63
<210> <211> <212> <213>	45 51 DNA unknown					
<220> <223>	primer					
<400> tcgggc	45 cccg ggctcgagtc	taacgttcgt	acggcccaag	aaatctagac	С	51
<210> <211> <212> <213>	46 27 DNA unknown		,			
<220> <223>	primer					
<400> ggagco	46 atac agagagcgat	ttattcg				27
<210> <211> <212> <213>	47 27 DNA unknown					
<220> <223>	primer					
<400> ttgaaa	47 tcga attaataagt	ctggatg				27
<210> <211> <212> <213>	48 24 DNA unknown					
<220> <223>	primer					
<400> tactgo	48 gcgg gcgctgctaa	agcg				24
<210> <211> <212> <213>	49 24 DNA unknown					
220s						

<223>	primer	NED ESE.SIES. CAC	
<400> aatttc	49 tgct ctcgcctgcc	gggc	24
<210> <211> <212> <213>	50 24 DNA unknown		
<220> <223>	primer		
<400> ccagtc	50 catg atcttctgaa	cgcc	24
<210> <211> <212> <213>	51 24 DNA unknown		,
<220> <223>	primer		
<400> agggaga	51 atcg acagagatca	tcgc	24
<210> <211> <212> <213>	52 58 DNA unknown		
<220> <223>	primer		
<400> tggccg	52 ggat ccggaggttt	aaaatatgca tccgatcgcc agcactgaaa ctcgccgc	58
<210> <211> <212> <213>	53 47 DNA unknown		
<220> <223>	primer		
<400> ttgccg	53 catg cctcacgagg	cagattccgg aatctcacag aagagtc	47
<210> <211> <212> <213>	54 27 DNA unknown	•	
<220> <223>	primer		
<400>	54		

ggagcca	atac agagagcgat	ttattcg	NED-232.3123. CAC	27
<210> <211> <212> <213>	55 27 DNA unknown			
<220> <223>	primer			
	55 tcga attaataagt	ctggatg		27
<210> <211> <212> <213>	56 24 DNA unknown			
<220> <223>	primer			
<400> ctttccc	56 cgta cttacaccga	tgcc		24
<211> <212>	57 24 DNA unknown			
<220> <223>	primer			
<400> tcctcga	57 atct tgggcgaacg	ggcc	•	24